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THE EFFECT OF TEMPERATURE AND EXPOSURE TIMES ON THE SURVIVAL OF *SITOPHILUS ORYZAE* L. IN AN ON-SITE GENERATED CONTROLLED ATMOSPHERE

A. CONTESSI,[1]* N. BALDASSARI[2] AND M. MAGGIOLI[2]

[1]*Plant Protection Services of the Emilia-Romagna region, 48100 Ravenna Italy*
*[*e-mail: sfr.ravenna@libero.it]*
[2]*Agricultural College, Faculty of Cesena, University of Bologna, Bologna, Italy*

ABSTRACT

This experiment involved the use of adult insects of the rice weevil *Sitophilus oryzae* L. in all phases of development. Three airtight mini-cells were placed inside a container with an internal temperature of 20±1ºC. The CA introduced into the mini-cells was produced directly on site using the Ecogen 8000 equipment. This instrument is an inert gas generator which produces a mixture of N_2 and CO_2 (<14%) with an oxygen content < 1%. Every sample to be tested consisted of a nylon net bag containing 100 g of infested durum wheat to which 50 live adults of *S. oryzae* were added. The study involved the treatment of *S. oryzae* in a CA at three different temperatures (20, 25 and 30ºC), for various lengths of time, which were established on the basis of results obtained in previous experiments. The effectiveness of the treatment was measured by sieving the content of each bag taken in a pre-selected order, counting the number of dead and alive adults, and then comparing the results with the control samples held at temperatures of 20, 25 and 30ºC. Mortality counts were carried out immediately after the treatment and after 30 days, during which period the samples were kept in a thermostatic chamber at 30ºC. The CAs produced on site, if used at high temperatures, have proved to be an effective alternative to the use of toxic gases; on the other hand at medium-low temperatures they appeared to be less effective because, although they eliminated the adults, insects in the early development stages survived to various degrees.

INTRODUCTION

The need to protect stored foodstuffs from attack by insect pests coupled with the desire to maintain high quality, that includes prevention from contamination with toxic pesticides, has led to a growing interest in identifying rational preventative methods together with innovative disinfestation technologies. Currently, the most widely used preventative method in the stored grain sector in Italy is the use of contact insecticides, this being considered as the most economic and easiest procedure to apply. Companies looking for higher quality normally use fumigation with toxic gases, in particular, phosphine (PH_3). This, however, may leave toxic residues and over time can lead to resistance phenomena. Strains

resistant to PH_3 have already been identified in numerous insect populations (Adler, 1997a; Conyers and Bell, 1997; Price, 1986; Contessi, 1999).

Controlled atmospheres (CAs) are currently one of the most promising alternative systems to these conventional methods, because they do not affect seed germination or the nutritional, organoleptic and technological properties of the treated grain. There are various methods of applying this technology (Adler *et al.*, 1997). One of these consists of reducing the oxygen (O_2) levels to less than 1% (hypoxia or anoxia) and increasing the carbon dioxide (CO_2) concentration to above 10% (hypercarbia). This technique may be associated with the addition of heat, to increase insect metabolism, thereby reducing treatment times (Adler, 1997b).

An efficient methodology for applying this method involves combustion of propane gas which enables O_2 levels of less than 1% and CO_2 levels of 12-13% to be obtained (Bell *et al.*, 1993; Bell *et al.*, 1997). Recently, equipment has become available on the Italian market, which can produce CAs directly *in situ* without the need to transport the gases to the warehouse.

The study carried out here aimed to test the efficiency of this recent innovative system, by assessing the action of the CAs at various temperatures in relation to exposure time. The test insect was, the rice weevil, *Sitophilus oryzae* L. (Coleoptera, Curculionidae), one of the commonest insects that infest stored grains. Furthermore, this species is reported in the literature to be among the most resistant to anoxia, both in the pupal and adult stages (Flinn and Hagstrum, 1996; Mbata and Reichmuth, 1997).

MATERIALS AND METHODS

Biological material

Adults and immature stages of *S .oryzae* developing within wheat grains were used in the experiments. The stock culture reared on wheat was maintained in a thermostatically controlled chamber 27±1ºC.

A total of 50 test samples were prepared in fine-mesh nylon bags, each containing 50 g of infested grains, 50 g of healthy grain and 50 adult insects. The bags were closed and labelled (Fig. 1).

Equipment

The experiment was performed inside three gas-proof mini-cells, placed inside a heat-insulated container maintained at 20 ±1°C. Each mini-cell was ~ 0.20 m^3 in volume, with a circular opening of ~ 20 cm, diameter sealed with an airtight plug and a valve for releasing excess gas (Fig. 2. The different temperatures inside the mini-cells were kept constant and uniform using thermostatically controlled heating elements and a ventilation system.

The CA supplied to the mini-cells was produced by an ECOGEN 8000 unit (Fig. 3), produced by ISOLCELL, Laives (Bolzano). This unit is an inert gas generator, which can produce a mixture containing approximately 86% N_2 and 14% CO_2, with an O_2 level of less than 1%, by combustion of O_2 in the air with methane or propane gas.

Fig. 1. Fine-mesh nylon bags employed.

Fig. 2. Gas-proof mini-cells employed for experiment.

The generator has the following parts: suction unit, generation unit, command and control unit, heat dissipation unit, dehumidifying unit, purification unit, and recording unit.

Fig. 3. Inert gas generator employed for experiment.

Test procedure

The protocol consisted of exposing *S. oryzae* in a CA at temperatures of 20, 25 and 30°C and for various durations (Table 1), established on the basis of results obtained in previous applications of this method (Contessi, 1998).

Before the experimentation began, the three mini-cells were filled to about one-third volume, with non-infested durum wheat. The test samples were then placed within the wheat. The control samples, prepared in the same way as the test samples, were held in thermostatically controlled chambers at the same

temperatures as the mini-cells. The gas produced by the CA equipment was conveyed by a compressor to a tank with a capacity of 10,000 L from where it was supplied to each of the three mini-cells. The gas was supplied initially at the maximum rate of 5 L/min using a flow-meter. Once the O_2 level had dropped below 1% and the CO_2 rose to 14%, the flow rate was reduced to approximately 1 L/min, which remained the operating rate for the entire process.

When test samples were collected, the CA flow rate was increased to the maximum during sampling, to prevent entry of ambient air into the mini-cells. A centralised monitoring system continually controlled and recorded O_2 and CO_2 concentrations in each cell. During the entire exposure period, O_2 values were always lower than 1% and the CO_2 values were between 11 and 14%. The moisture content of the grain inside the three mini-cells is indicated in Table 2.

TABLE 1
Experimental protocol for exposure of *Sitophilus oryzae* to a CA produced by an inert gas generator containing ~86% N_2, 14% CO_2 and <1% O_2

	Mini-cell temperature		
	20°C	25°C	30°C
Exposure duration (days)	28	20	14
Insect sampling dates	from day 21 to 28	from day 14 to 20	from day 8 to 14
Number of samples treated	16	14	14
Number of control samples	2	2	2

Assessment of treatment efficacy

To assess the optimal duration of the treatment in relation to the operating temperatures, two of the treated samples were collected every day and the numbers of live adults were counted.

In addition, to assess the efficacy of the treatments on the pre-adult stages the same samples were checked again, after being held for 40 days at 27°C, to enable adult emergence of surviving insects.

TABLE 2
Moisture contents of the treated grain inside the mini-cells

Temperature	Moisture content
20 °C	11.6 %
25 °C	11.3 %
30 °C	10.3 %

RESULTS

The results of the treatments against *S. oryzae* adults and immature stages using the on-site CA generator, at 3 temperatures for the different exposure times are given in Tables 3, 4 and 5 and in Fig. 4.

All the treatments carried out, including those at the lower temperatures and for the shorter exposure periods, led to 100% mortality of the adult insects. Data on the average number of live adults reared from each treated sample, after 40 days incubation, showed that at 30°C application of a CA, containing 11% N_2, 14% CO_2 and <1% O_2 caused complete mortality of all stages of *S. oryzae*, after 9 days of exposure. At 20°C, this result was not obtained for any of the exposure periods tested, while at 25°C the number of adults emerging from the incubated grain did not show a clear trend though in all cases, the insect survival of the treatments at 20 and 25°C was considerably less than that of the control.

TABLE 3
Effects of treatment at 20°C

Duration of treatment (days)	Average number of live adults (1st check)	Average number of live adults (2nd check)
21	0	9
22	0	7
23	0	3.5
24	0	6
25	0	4
26	0	3
27	0	2.5
28	0	2.5
Control	67	308

TABLE 4
Effect of treatment at 25ºC

Duration of treatment (days)	Average number of live adults (1st check)	Average number of live adults (2nd check)
14	0	7.5
15	0	4
16	0	3
17	0	1
18	0	0
19	0	1.5
20	0	0.5
Control	70	435

TABLE 5
Effect of treatment at 30ºC

Duration of treatment	Average number of live	Average number of live
8	0	0.5
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
Control	74	550

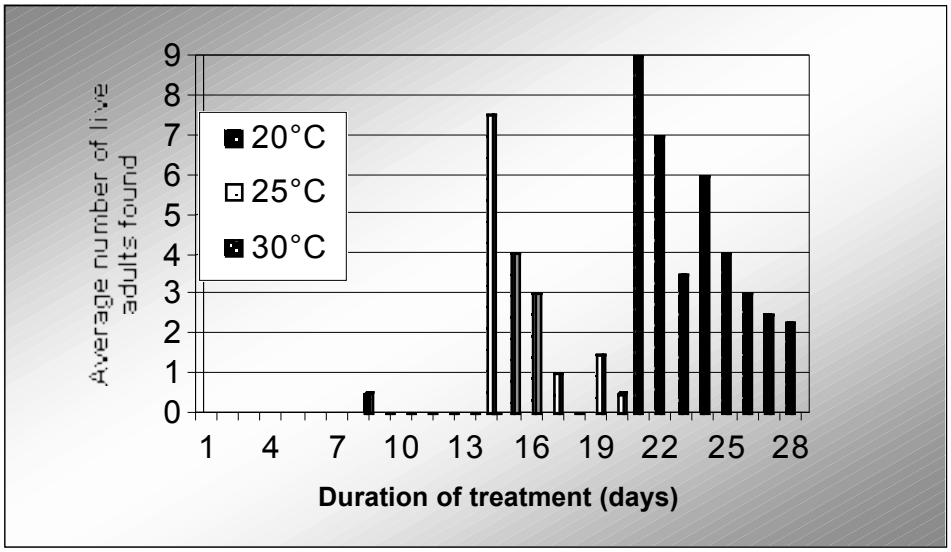

Fig. 4. Effect of the treatment with a CA containing <1% O_2 and 11-14% CO_2 on populations of *Sitophilus oryzae* in relation to temperature and duration of treatment.

CONCLUSIONS

There is a worldwide interest in the development of new techniques for preserving cereal grains, as alternatives to the chemical control methods currently employed. This has intensified into a search for more-environmentally friendly methods that do not leave residues on the treated grain and guarantee a higher-quality of raw material, while still maintaining a high level of insecticidal efficacy.

The experiments carried out here are, in agreement with data in literature (Adler *et al.*, 1997; Bell *et al.*, 1997), showing that the technique of on-site production of CAs, i.e. without the need to transport large quantities of gas, is a valid alternative to the use of contact insecticides or toxic gases for the disinfestation of stored cereals. In addition, the technology can also be adopted in incompletely sealed storage environments since the CA supplied through this process, creates a slight overpressure which prevents entry of O_2 from outside (Contessi, 1998).

However, these results also show that where the species is particularly tolerant to CAs, as in the case of *S. oryzae*, the critical factor is temperature. We showed that, at 30°C, less than 9 days were sufficient to ensure 100% mortality of the insects at all developmental stages. At 25°C, the same result required 18-20 days exposure and at 20°C, a 100% death rate was not obtained even after 28 days. Therefore, when species especially tolerant to anoxia are present, the technique must be applied to cereal bulks at temperatures above 25°C in order to obtain 100% mortality, of all stages within a reasonable exposure duration. In temperate climates, this probably means that treatment can only be commenced in early autumn.

Clearly, the technology to be chosen for the preservation of stored grain must take into account economic considerations; though the economic costs must also be assessed in terms of the quality of the product obtained. Studies in Australia have shown that the total cost of treatment using O_2 levels below 1% and CO_2 levels between 10 and 14% is higher than that of conventional PH_3 fumigation

but that it is still competitive when compared with methyl bromide (Allanson, 1997).

The increase in average cost per ton of cereal grain preserved using CAs produced on-site, is estimated at US$ 1-3/tonne, as compared to the cost of conventional methods, estimated at US$ 10-11/tonne (Zuppiroli, 1998); this may justify decisions to use this technology, even in economic terms, since a better quality product is obtained.

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